

Modulation of the human preadipocyte mitochondrial activity by beta-carotene*

Agnieszka Śliwa[✉], Joanna Góralska, Urszula Czech, Anna Gruca, Anna Polus, Barbara Zapala and Aldona Dembińska-Kieć

Department of Clinical Biochemistry, Jagiellonian University Medical College, Kraków, Poland

Increased ROS generation by the overload by metabolic substrates mitochondria paralleled by decrease of antioxidant activity are typical events found in metabolic syndrome and diabetes type 2. Metabolites of beta-carotene (BC) such as retinoic acid (RA), as well as low concentration of reactive oxygen species (ROS) modify the mitochondrial bioenergetic function. The aim of the study was to investigate the effect of beta-carotene on mitochondrial activity in human preadipocytes. BC used in concentrations, 10 or 30 μ M, decreased mitochondrial membrane potential, inhibited mitochondrial respiration and decreased cellular ATP content. We conclude, that BC, the known antioxidant may decrease oxidative phosphorylation capacity of mitochondria.

Key words: beta-carotene, preadipocytes, mitochondria, oxidative phosphorylation

Received: 14 October, 2011; **accepted:** 01 March, 2012; **available on-line:** 17 March, 2012

INTRODUCTION

Mitochondria play an essential role in cellular bioenergetics. Disturbances in mitochondrial metabolism play a role in aging as well as in acquired and inherited metabolic disorders (Borutaite, 2010). Abnormal mitochondrial function results in lipid droplets accumulation characteristic for insulin resistance, as cells require a balance between oxidative phosphorylation (OXPHOS) and dissipation of the proton gradient, to minimize damage of “glucolipotoxicity”-induced reactive oxygen species (ROS) generation (Bournat & Brown, 2010). Changes in mitochondrial biogenesis and function have been documented in the diabetes and metabolic syndrome (Ren *et al.*, 2010). Mitochondrial dysfunction in mature adipocytes has been linked to defects in fatty acid oxidation, secretion of adipokines and dysregulation of glucose homeostasis (Bournat & Brown, 2010).

In human beta-carotene (BC) is converted to retinoic acid (RA) which exert potent effects on cell differentiation, proliferation and fetal development (Kotake-Nara *et al.*, 2001; Palozza *et al.*, 2001). Several studies have proved that carotenoids may act as anti-oxidants or as pro-oxidants, depending on their concentration into the cells as well as on cell oxidative environment (Palozza, 1998). Last reports revealed that mitochondrial dysfunction leads to impaired lipid metabolism and/or oxidation of lipids, proteins, and mtDNA, which trigger the accumulation of TGs in the cytosol of preadipocytes, a process mediated through a decrease in fatty acid β -oxidation and an increase in lipogenesis (Wang *et al.*,

2010). Since the effect of this nutrient on metabolism of human preadipocytes is still not well recognized, this work was aimed to investigate the influence of BC on mitochondrial bioenergetic functions, in immortalized human Chub-S7 preadipocytes.

MATERIALS AND METHODS

Reagents. All the reagents for cell culture were purchased from Sigma Aldrich Steinheim (Germany) unless noted otherwise.

The HPLC grade beta-carotene was kindly provided for the study by the Roche Vitamins AG, Kaiseraugst, Switzerland.

Cells culture. The Chub-S7 (Nestec Ltd) cell line was derived from human subcutaneous adipose tissue by co-expression of human telomerase reverse transcriptase and papillomavirus E7 oncoprotein (HPV-E7) genes (Darimont *et al.*, 2003). Confluent Chub-S7 cells were cultured in DMEM/Ham's F-12 medium supplemented with 10% Fetal bovine serum (Gibco). The cells were incubated with three different concentrations of beta-carotene (3 μ M; 10 μ M and 30 μ M) in 5% CO₂ at 37°C for 24 hours. Control cells were incubated with an appropriate concentrations of solvent (0.05% final concentration of THF/ethanol).

Mitochondrial membrane potential ($\Delta\Psi$ m). Mitochondrial membrane potential was analyzed by MitoProbe™ JC-1 Assay Kit for flow cytometry (Molecular Probes). Mitochondrial depolarization was indicated by a decrease in the red/green fluorescence intensity ratio.

Mitochondrial respiration. The function of the respiratory transport chain was analyzed by high-resolution respirometry in Oxygraph-2k respirometer (Oroboros Instruments) according to the phosphorylation control protocol for intact cells (Garedeu *et al.*, 2010). The protocol included in sequence: (i) a 10-min period of ROUTINE respiration, reflecting the aerobic metabolic activity under cellular routine conditions (ii) the oligomycin (final concentration 2 μ g/ml)-inhibited LEAK respiration, which is caused mainly by compensation for the proton leak after inhibition of ATP synthase; (iii) the FCCP (p-trifluoromethoxy carbonyl cyanide phenyl hydrazone) titration, which yields the maximum stimulated respiration, as a measure of electron transport system capacity

✉ e-mail: agasliw@interia.pl

*Presented at the 16th International Symposium on Carotenoids, 17–22 July, 2011, Kraków, Poland

Abbreviations: ATP, adenosine-5'-triphosphate; BC, beta-carotene; OXPHOS, oxidative phosphorylation; RA, retinoic acid; ROS, reactive oxygen species; TGs, triglycerides; $\Delta\Psi$ m, mitochondrial membrane potential.

of uncoupled mitochondria. Inhibitor and the uncoupler applied in this protocol are freely permeable through the intact plasma membrane and do not require cell membrane permeabilization (Renner *et al.*, 2003). The rates of respiration was calculated as time derivative of oxygen flux rates per million cells.

ATP assay. The intracellular ATP content was measured using ATPlite™ Luminescence ATP Detection Assay System (Perkin Elmer). Results were calculated with Magellan6 software as nmol ATP, and then adjusted for protein content (measured by Lowry method), and reported as nmolATP/mg of protein.

RESULTS

Mitochondrial membrane potential ($\Delta\Psi_m$)

The significant decrease of $\Delta\Psi_m$ in Chub-S7 cells after incubation with 10 μM as well as 30 μM of BC was observed (Fig. 1) and the effect was stronger at the higher concentration of BC. At the lower concentration of BC (3 μM) we did not observe any influence on $\Delta\Psi_m$ in Chub-S7 cells.

Mitochondrial respiration

A dose-dependent inhibition of routine respiration was observed after incubation of Chub-S7 cells with beta-carotene (Fig. 2). At the lowest concentration (3 μM) it was only tendency, when at the higher concentrations of BC the effect was significant. Additionally, BC (especially at 10 μM concentration) reduced the oligomycin-inhibited LEAK respiration (Fig. 3), which is caused mainly by compensation for the proton leak after inhibition of ATP synthase. The maximum stimulated respiration (the measure of electron transport system capacity of uncoupled mitochondria) represent as ETS was also decreased after incubation with BC (Fig. 3).

Intracellular ATP content

Incubation with higher concentrations of BC, especially 10 μM , decreased the cellular ATP content (Fig. 4). After incubation with 3 μM of BC we did not observe any significant effect.

DISCUSSION

The reported basal concentrations of beta-carotene in human serum does not exceed 1 $\mu\text{mol/l}$ and BC supplementation increased it by about 10–15-fold (Dembńska-Kieć *et al.*, 2005; Dulińska *et al.*, 2005). In our experiments, we used “physiological” up to 30 μM BC concentrations, which is commonly used (Dembńska-Kieć *et al.*, 2005; Sacha *et al.*, 2005; Kieć-Wilk *et al.*, 2009).

Carotenoids may act as anti-oxidants or as pro-oxidants, depending on their concentration in the cells, cell type and cell oxidative environment (Palozza, 1998). High concentrations of carotenoids seem to enhance their pro-oxidant effects in biological systems (Palozza *et al.*, 2001). Beta-carotene can induce the release of cytochrome *c* from mitochondria and change mitochondria membrane potential in different tumor cells (Palozza *et al.*, 2004), what is in accordance with our results showing that BC significantly decreases mitochondrial membrane potential in human preadipocytes.

Carotenoids may undergo oxidation and form carotenoid-derived aldehydes (CDA), which can be toxic to

tissues. Kalariya *et al.* (2008) observed that CDA increase apoptosis in pigment epithelial cells. Early appearance of apoptotic changes in these cells were associated with change in $\Delta\Psi_m$. It is likely that CDA forms adducts with thiol groups of the mitochondrial proteins,

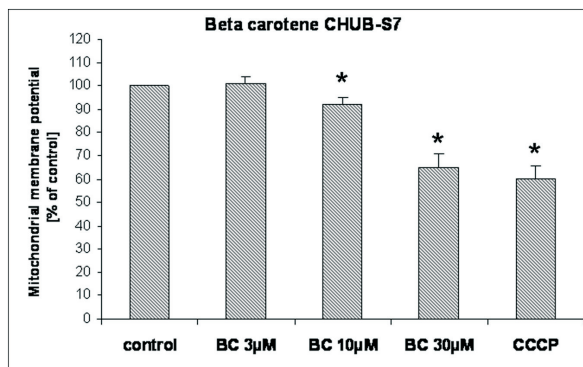


Figure 1. Effect of beta-carotene (BC) on mitochondrial membrane potential in Chub-S7 cells incubated with BC (3, 10, 30 μM) for 24h.

Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was used as positive control. Values are given as % of control \pm S.D. from 5 independent experiments. * $p < 0.05$ vs control.

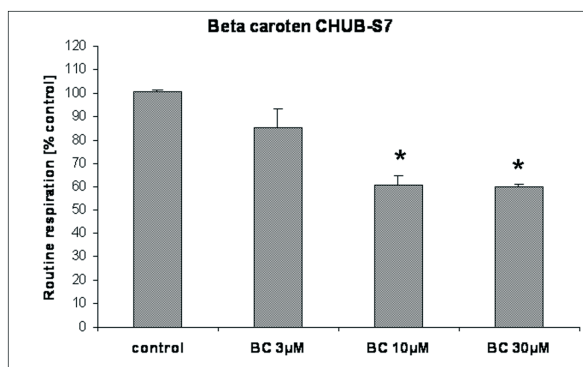


Figure 2. Influence of beta-carotene (BC) on mitochondrial routine respiration.

Cells were incubated with 3, 10, 30 μM of BC for 24 h. Values are given as % of control \pm S.D. from 5 independent experiments. * $p < 0.05$ vs control.

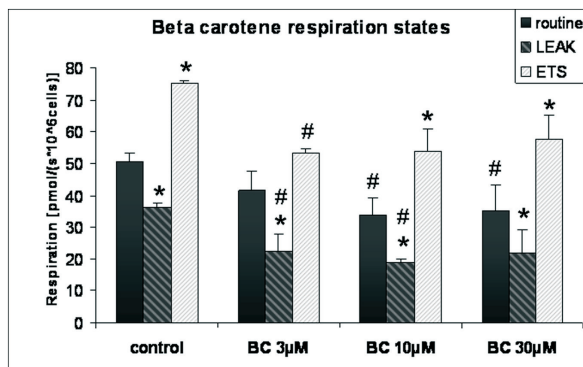


Figure 3. Mitochondrial respiration was analyzed by high-resolution respirometry.

routine, routine respiration; LEAK, the oligomycin (final concentration 2 $\mu\text{g/ml}$)-inhibited respiration, ETS, electron transport system capacity of uncoupled mitochondria. Values are given as pmol/(s \times 10⁶cells) \pm S.D. from 5 independent experiments. * $p < 0.05$ vs routine respiration for each group; # $p < 0.05$ vs control.

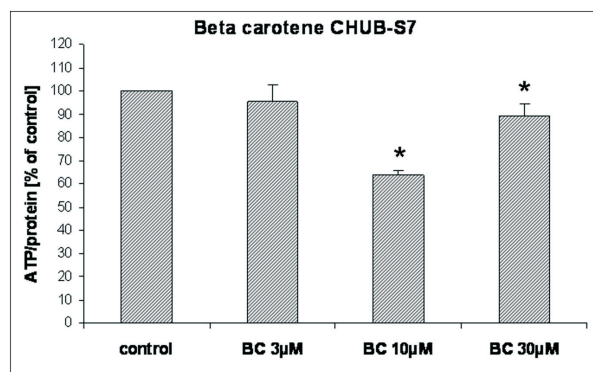


Figure 4. Changes in ATP content in Chub-S7 cells after 24 h incubation with beta-carotene (BC) (3, 10, 30µM). Values are given as % of control \pm S.D. from 5 independent experiments * $p < 0.05$ vs control.

such as the permeability transition pore and release of cytochrome *c*, which has been proposed to trigger apoptosis (Takeyama *et al.*, 2002).

Carotenoid cleavage products — retinal as well as β -ionone — were demonstrated to increase oxidative stress in isolated rat liver mitochondria by impairing mitochondrial function (Siems *et al.*, 2002). We have demonstrated, that BC decreases mitochondrial routine respiration and reduces the oligomycin-inhibited LEAK respiration, which is caused mainly by compensation for the proton leak after inhibition of ATP synthase. Siems *et al.* (2002) reported that carotenoid cleavage products strongly inhibit the ADP-induced increase in respiration in a concentration-dependent manner. Impairment of adenine nucleotide translocator may be responsible for the decrease in respiration (Siems *et al.*, 2005). This observation is in disagreement with our results because in our experiments additional we observed decrease $\Delta\Psi_m$, that suggests existence of other mechanism.

Carotenoids accumulating in hepatocytes induce oxidative stress, change activity of manganese superoxide dismutase, and cause mitochondrial dysfunction, by reduction of ADP-dependent respiration rates as well as depolarization of mitochondrial membranes (Amengual *et al.*, 2011). Carotenoid metabolites strongly inhibit state 3 respiration of rat liver mitochondria (Siems *et al.*, 2009). The decrease of ATP generation in skeletal muscles by the other antioxidant: Vit C in athletics has been also reported (Gomez-Cabrera *et al.*, 2008).

Thus the metabolic effect of beta-carotene, which is widely used as dietary supplement, is still not completely recognized in different cell types. We conclude that it may impair mitochondrial function of human preadipocytes by decreasing mitochondrial membrane potential, inhibition of mitochondrial respiration and ATP generation. The presented data suggest that potential mechanism of beta-carotene-induced apoptosis could be related with mitochondrial pathway.

Acknowledgements

This work was Supported by EU FW7 LIPIDOMIC-NET 202272; K/ZBW/000577.

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